MAIL STOP APPEAL BRIEF-PATENTS
Attorney Docket: 25871

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:

	THOMPSON et al.	Conf No.:	6428
Serial No:	10/767,431	Examiner:	E. Arnold
Filed:	January 30, 2004	Art Unit:	1616

For: **METHOD OF TREATING CANDIDA ISOLATES**

APPEAL BRIEF

This is an appeal to the Board of Patent Appeals and Interferences from the decision of Examiner Ernst V. Arnold, mailed April 10, 2007, rejecting claims 1-27. Appellants filed a Notice of Appeal on September 7, 2007 making an Appeal Brief due on or before November 7, 2007.

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REAL PARTY IN INTEREST

The real party in interest is the assignee, DRUGTECH CORPORATION, which is a wholly owned subsidiary of KV Pharmaceutical Company.

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

STATUS OF CLAIMS

Claims 1-27 are pending. Claims 1-27 have been rejected, the rejection thereof is hereby appealed. The claims appealed are reproduced in the Appendix appearing in this paper on pages 31-40.

STATUS OF AMENDMENTS

No claim amendments have been entered since the Official Action dated April 10, 2007 was mailed.

SUMMARY OF CLAIMED SUBJECT MATTER

The presently claimed subject matter is directed to methods of treatment for specific non-*albicans* species of *Candida* isolates using antimycotic delivery systems. These systems are suitable for use in the vaginal cavity. The presently claimed subject matter is additionally directed to methods utilizing preparations demonstrating a controlled, extended or sustained release of an active and/or therapeutic agent requiring a minimal number of administrations to achieve efficacy upon administration of the delivery system.

In particular, pending independent claim 1 is directed to a method for the local treatment of a vulvovaginal candidiasis, which comprises: treating said vulvovaginal candidiasis condition caused by a species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae* by applying to the vaginal tissue of a human a formulation comprising: about 35 to about 45% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1% w/w methylparaben; about 0.001 to about 1% w/w propylparaben; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate; and wherein the treatment is a single dose treatment. Support for this claim may be found throughout the specification and claims as originally filed, for example, at

page 6, lines 11-23.

Further, independent claim 6 is directed to a method for the treatment of a vaginal fungal condition, which comprises: administering a single dose composition comprising about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01 to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate; wherein the vaginal fungal condition is a vulvovaginal candidiasis condition caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*, and wherein the ratio of polyglyveryl-3-oleate to glyceryl monoisostearate is about 1:0.1-10. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 7, lines 1-13.

Additionally, independent claim 9 is directed to a method for the treatment of an unidentified vulvovaginal fungal condition, which comprises: administration to said fungal condition a bioadhesive, single dose treatment formulation comprising from about 0.500 to about 5.000% w/w butoconazole nitrate; and wherein the unidentified vulvovaginal fungal condition is caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*,

parapsilosis, *krusei*, and *lusitaniae*. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 7, lines 14-20.

Furthermore, independent claim 17 is directed to a method for the treatment of a fungal condition, which comprises: applying to a vulvovaginal candidiasis condition caused by a member selected from the group consisting of *Candida dubliniensis*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, mycelial *Candida*, *Candida krusei*, and *Candida lusitaniae* and mixtures thereof of a treatment comprising: about 35 to about 45% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1% w/w methylparaben; about 0.001 to about 1% w/w propylparaben; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 7, line 14 to page 8, line 10.

In addition, independent claim 19 is directed to a method for the local treatment of an unidentified vaginal fungal condition comprising: administering a single administration of a composition consisting essentially of: about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01

to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate; and wherein the administration is to a vulvovaginal candidiasis condition caused by any member selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae*. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 8, line 11-22.

Moreover, independent claim 22 is directed to a method for the treatment of a fungal condition, comprising: treating a candidiasis condition caused by a species selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* by applying to the vaginal tissue a multiphase formulation in a single dose; wherein the multiphase formulation comprises: a hydrophilic phase, which comprises: about 38 to about 40% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate; and a hydrophobic phase which comprises about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about

0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1.000% w/w methylparaben; and about 0.001 to about 1% w/w propylparaben. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 9, lines 1-14.

Finally, independent claim 24 is directed to a method for the treatment of an unidentified vulvovaginatis condition comprising: treating a condition caused by a species of *Candida* selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* by applying to the vaginal tissue a multiphase formulation in a single dose to provide a *Candida* species kill rate of about 50 to about 100% for a period of at least about 4 days. Support for this claim may be found throughout the specification and claims as originally filed, for example, at page 9, lines 15-20.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Whether claims 9 and 24-27 are patentable under 35 U.S.C. § 102(b) as being anticipated by Brown et al. (*The Journal of Reproductive Medicine*, 44(11) 933-938, 1999), in view of Stedman's Medical Dictionary (24th Edition, 1982, page 334).

Whether claims 1-27 are unpatentable under 35 U.S.C. 103(a) as being obvious over Riley in U.S. Patent No. 5,266,329, in view of Brown et al. (*The Journal of Reproductive Medicine*, 44(11) pp. 933-938, 1999), Garg (*Pharmaceutical Tech. Drug Delivery*, 2001, pp. 14-24), Droegemueller et al. (*Obstet Gynecol*, 64(4), pp. 530-534, 1984) and Chen in U.S. Patent No. 2,267,985.

ARGUMENTS

I. The rejection of claims 9 and 24-27 under 35 U.S.C. § 102(b) as being anticipated by Brown et al. in view of Steadman's Medical Dictionary.

Claims 9 and 24-27 are rejected under 35 U.S.C.§102(b) as being anticipated by Brown et al., et al., *Journal of Reproductive Medicine*, in view of Stedman's Medical Dictionary ("Brown et al." and "Stedman's," respectively).

Appellants respectfully traverse the rejection of claims 9 and 24-27 under 35 U.S.C. §102(b) and request reconsideration and withdrawal thereof.

The test for anticipation is whether each and every element as set forth is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California,* 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); MPEP § 2131. The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.,* 9 USPQ2d 1913, 1920 (Fed. Cir. 1989); MPEP §2131. The elements must also be arranged as required by the claim. *In re Bond,* 15 USPQ2d 1566 (Fed. Cir. 1990).

"For a prior art reference to anticipate a claim, the reference must disclose each and every element of the claim with sufficient clarity to prove its existence in the prior art...The reference must describe the applicant's claimed invention sufficiently to have placed a person of ordinary skill in the field of the invention in possession of it." *See In re Spada,* 911 F.2d 705 (Fed. Cir. 1990). Although the disclosure requirement presupposes the knowledge of one skilled in the art, that

presumed knowledge does not grant a license to read into the prior art reference teachings that are not there. *Id.*

"Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *See In re Oelrich,* 666 F.2d 578, 581 (CCPA 1981) (Emphasis Added). "Occasional results are not inherent." *See Mehl/Biophile International Corp. v. Milgraum,* 192 F.3d 1362 (Fed. Cir. 1999). "A reference includes an inherent characteristic if that characteristic is the natural result flowing from the reference's explicitly explicated limitations." *See Continental Can Company USA, Inc., supra.*

Claim 9 is directed to a method for the treatment of an unidentified vulvovaginal fungal condition, which comprises: administration to said fungal condition a bioadhesive, single dose treatment formulation comprising from about 0.500 to about 5.000% w/w butoconazole nitrate; and wherein the unidentified vulvovaginal fungal condition is caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

Claim 24 is directed to a method for the treatment of an unidentified vulvovaginatis condition comprising: treating a condition caused by a species of *Candida* selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* by applying to the vaginal tissue a multiphase formulation in a single dose to provide a *Candida* species kill

rate of about 50 to about 100% for a period of at least about 4 days. Claims 25-27 all depend from claim 24.

Appellants submit that Brown et al. in view of Stedman's does not expressly or inherently teach each and every element of the claims 9 or 24-27 as required for anticipation under 35 U.S.C. § 102(b).

Brown et al. is directed to a comparison of the safety and efficacy of (1) a single vaginal dose of butoconazole nitrate 2% sustained release cream with (2) a seven day schedule of miconazole nitrate 2% vaginal cream in the treatment of vulvovaginal candidias caused by *C. albicans*. See, Objective, Introduction, Patient Selection, and Results: Microbiological Cure Rate sections. Steadman's simply provides a definition for "cream" as meaning a semisolid emulsion of either the oil-in-water type, ordinarily intended for topical use.

Brown et al. in view of Stedman's does not teach administration of a bioadhesive single dose treatment formulation comprising from about 0.500 to about 5.000% w/w butoconazole nitrate to a vulvovaginal fungal condition caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae* as recited in claim 9. Additionally, Brown et al. in view of Stedman's does not teach treating a **condition caused by a species of *Candida* selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae*** by applying to the vaginal tissue a multiphase formulation in a single dose to provide a *Candida* species kill rate of about 50 to

about 100% for a period of at least about 4 days as recited in claim 24.

However, at page three of the Official Action, the following is set forth:

> Brown et al. clearly discloses on page 934, lower right column that butoconazole nitrate has a "broad antifungal spectrum consistently showing high activity against the most important eight non-*albicans* Candida species" and "inhibiting the growth of C. *albicans* as well as the non-*albicans* pathogens species." Furthermore, Brown et al. treated 101 patients with butaconazole nitrate and stated that 10-20% of the cases are non-*albicans* Candida species and *C. glabrata* is the second most frequently encountered species (Page 934, left column and page 936, Table 1). Thus Brown et al. disclose treatment of vulvovaginal fungal caused by at least *C. glabrata*. Simply because Brown et al. did not test for the presence of other species of Candida, does not mean that there were not present especially in light of the [asserted] fact that 10-20% for the cases are caused by non-*albicans* Candida species. The treatment of other Candida species in the method of Brown et al. is inherent in the method. *See* Official Action at page 3.

Appellants respectfully disagree. It is asserted that of the 101 patients tested in Brown et al., at least 10-20% of the cases were infected with non-*albicans Candida* species. It is further asserted that "Brown et al. disclose treatment of vulvovaginal fungal caused by at least *C. glabrata*." *See* Supra. This line of reasoning is used to draw a nexus between the study preformed in Brown et al. with background information provided by the authors of Brown et al. This line of reasoning is incorrect. If one refers to the passage in Brown et al. regarding patient selection, one finds the following:

> Healthy, nonpregnant women with clinical signs and symptoms of vulvovaginal candidiasis participated in the study. KOH vaginal smears and samples of vaginal fluid were cultured on Sabouraud's and Micosel media (BBL Microbiology Systems, Cockeysville, Maryland) and confirmed or excluded the clinical impression of

> vulvovaginal candidiasis. **Two microbiologic laboratories identified blindly the presence of *C albicans*.** Examination of wet smears showing trichomonads or clue cells associated with bacterial agents excluded patients from enrollment. A test for gonadotropin was performed prior to admission to the study. (Emphasis Added). *See* Brown et al. at page 935, column 2, paragraph 3.

Hence, the 101 patients studied in Brown et al. were strictly chosen on the basis of exhibiting the presence of *C. albicans*, and not just for exhibiting symptoms of vulvovaginal candidiasis alone. In fact, it would appear those women with **symptoms** of vulvovaginal candidiasis alone, who did not test positive for *C. albicans*, would have been excluded on that fact alone. Nowhere in Brown et al. is it either expressly or inherently taught that any of the 101 patients tested positive for a **condition caused by a species of *Candida* selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* or that the same could be treated**.

According to the holding of *In re Oelrich,* "**Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient**." *See supra, In re Oelrich.* Although Brown et al. teaches that vulvovaginal Candidiasis is **caused by *C. albicans*** as well as non-albicans pathogenic species, Brown only teaches that the single-dose butoconazole is **effective in treating *C. albicans,* alone**. *See* Brown at page 934 and page 936, second paragraph.

Futher, Brown et al. teaches that *C. glabrata* "is less susceptible to standard treatment." *See* Brown et al. at page 934, lower left column. Hence, Brown et al. does not teach any effective treatment of *C. glabrata*. Brown et al. only describes, firsthand, the treatment of *C. albicans*.

With regard to second hand knowledge described in Brown et al., at page 934, lower right column, "that butoconazole nitrate has a broad antifungal spectrum consistently showing high activity against the most important eight non-*albicans Candida* species" and "inhibiting the growth of *C. albicans* as well as the non-*albicans* pathogens species." However, Appellants note, as footnoted by Brown et al., the basis for this passage comes from a previously published article, Lynch, ME, Sobel, JD: *Comparative in vitro activity of antimcotic agents against pathogenic vaginal yeast isolates*, J Med Vet Mycol (1994), 32:267-274 ("Exhibit 1"), provided herewith for the Examiner's convenience.

Appellants respectfully submit that the treatments defined by the presently pending claims based on *in vivo* studies are distinguishable from the *in vitro* susceptibility noted by Lynch and Sobel. Further, as admitted by Lynch and Sobel, the results of their tests show that more testing in the area must be performed on non-*albicans* Candida. As evidence of the foregoing, the last paragraph at page 273 of Lynch and Sobel, states:

> **It is important to emphasize that *in vitro* susceptibility cannot always be extrapolated into predicting *in vivo* activity or clinical success even when an agent is applied locally resulting in high concentrations of the antifungal agent**. There have been relatively few published studies comparing *in vitro*

> activity of azoles against non-C. *albicans* species and clinical outcome in animal models or in patients. It is our experience however, that clinical cases of *C. glabrata* and *S. cervisiae* vaginitis respond less well to the majority of available therapies. **Since a successful animal model of *C. glabrata* vaginitis has not been established**, most of our information is based upon limited experience with clinical *C. albicans* vaginitis. Nevertheless, in two studies, in two studies, *in vitro* sensitivity tests performed on *C. glabrata* and *S. cerevisiae* were useful in predicting clinical failure with TER and FLU and moderate success with CLO [9, 13]. **The role of cidal activity of antifungal agents in determining the outcome of fungal vaginitis, especially when caused by species other than C. *albicans*, is unknown.** This may be a critical factor in determining, not the immediate outcome of therapy in alleviating symptoms, but the relapse and later reccurence rate, particularly of *C. glabrata* vaginitis. **This study identifies the need for futher investigation on the role of BUTO and ITRA in experimental and clinical vaginitis,especially when caused by non-*albicans* *Candida* species**. (Emphasis Added). *See* Lynch and Sobel at page 273.

Therefore, Appellants submit, Brown et al. **do not disclose** an effective treatment for a vulvovaginitis condition **caused by** a non-*albicans* Candida selected from the group consisting *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and C. lusitaniae with a single dose **treatment** of butoconazole. As established above, "**Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient**." Therefore, Appellants submit Brown et al. does not expressly or inherently anticipate the presently pending claims.

Accordingly, Appellants submit Brown et al. in view of Steadman's does not teach each and every element of claims 9 and 24-27, as required for anticipation under 35 USC § 102(b). Therefore, Appellants respectfully request that this rejection be reconsidered and withdrawn.

II. The rejection of claims 1-27 under 35 U.S.C. 103(a) as being unpatentable over Riley in U.S. Patent No. 5,266,329, in view of Brown et al., Garg, Droegemueller et al. and Chen in U.S. Patent No. 2,267,985.

Claims 1-27 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Riley in U.S. Patent No. 5,266,329 (the '329 patent), in view of Brown et al., Garg, Droegemueller et al. and Chen in U.S. Patent No. 2,267,985 (the '985 patent).

Appellants respectfully traverse the rejection of claims 1-27 under 35 U.S.C. §103(a). A *prima facie* case of obviousness has not been established with respect to the '329 patent, in view of Brown and Garg, Droegemueller et al. and the '329 patent for the reasons set forth below.

To establish a *prima facie* case of obviousness, the PTO must satisfy three requirements. First, as the U.S. Supreme Court very recently held in *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007), "a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions. ...it [may] be necessary for a court to look to interrelated teachings of multiple patents; the effects of demands known to the

design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. ...it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does... because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." (*KSR*, *supra*, slip opinion at 13-15.) Second, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc. v. Chugai Pharm. Co.*, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991). Lastly, the prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

Further, according to MPEP § 2141, "Patent examiners carry the responsibility of making sure that the standard of patentability enunciated by the Supreme Court and by the Congress is applied in each and every case. The Supreme Court in *Graham v. John Deere*, 383 U.S. 1, 148 USPQ 459 (1966), stated:

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the

> pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquires may have relevancy...." *See* MPEP § 2141(1).
>
> Accordingly, the four factual inquiries determining nonobviousness are:
>
> (A) Determining the scope and contents of the prior art;
>
> (B) Ascertaining the differences between the prior art and the claims in issue;
>
> (C) Resolving the level of ordinary skill in the pertinent art; and
>
> (D) Evaluating evidence of secondary considerations. *Graham v. John Deere*, 383 U.S. 1, 148 USPQ 459 (1966); Followed by *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007).

It is submitted that a *prima facie* case of obviousness has not been established according to the factors enumerated by Supreme Court in *Graham v. John Deere*.

a. Impermissible Hindsight

Appellants respectfully submit that the scope and contents of the prior art has been incorrectly determined. According to MPEP § 2141.01(III), content of the prior art is determined at the time the invention was made to avoid hindsight. The requirement "at the time the invention was made" is to avoid impermissible hindsight. "It is difficult but necessary that the decisionmaker forget what he or she has been taught . . . about the claimed invention and cast the mind back to

the time the invention was made, to occupy the mind of one skilled in the art who is presented only with the references, and who is normally guided by the then-accepted wisdom in the art." *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303, 313 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

Appellants respectfully submit the teachings of Brown et al. as evidenced by the additional publications of J.D. Sobel, demonstrate that Brown et al., were unaware of a viable method for effectively treating the claimed non-*albicans* *Candida* species. Examples of Sobel's publications include: Sobel, JD, "Treatment of vaginal Candida infections," *Expert Opin Pharmocother*, 3(8): 1059-65, Aug 2002 ("Exhibit 2"); Moosa, MY and Sobel JD, "Non-*albicans* Candida infections in patients with hematologic malignancies," *Semin Respir Infect.*, 17, (2): 91-8, Jun 2002 ("Exhibit 3"); Sobel et al., "Treatment of Complicated Candida vaginitis: comparison of single and sequential doses of fluconazole," *Am J Obstet Gynecol.*, 185(2): 363-9, Aug 2001 ("Exhibit 4"); Sobel JD, "Antimicrobial Resistance in Vulvovaginitis," *Curr Infect Dis Rep*, 3(6): 546-549, Dec 2001 ("Exhibit 5"), abstracts of each are attached hereto for convenience. The attached Sobel publications, published subsequent to Brown et al., relate to treatment of the different *Candida* species using various active agents in order to determine efficacy. Such research efforts clearly show that much remained unknown at the time Brown et al. was written. Therefore, the assertion that Brown et al. disclose each and every limitation of the subject

pending claims is only possible when viewed with impermissible hindsight.

The state of the art at the time the subject application was filed would not consider the use of a bioadherant, single dose treatment for a vulvovaginitis condition caused by non-*albicans* species of *Candida*; specifically, *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae*. According to Lynch and Sobel, "[t]here have been relatively few published studies comparing *in vitro* activity of azoles against non-*C. albicans* species and clinical outcome in animal models or in patients." *See* Lynch and Sobel at page 273. Concluding the article, Lynch and Sobel state, "[t]his study identifies the need for further investigation on the role of BUTO and ITRA in experimental and clinical vaginitis, especially when caused by non-*albicans* Candida species."

As additional evidence that the state of the art at the time the subject application was filed would not consider the use of a bioadherant, single dose treatment for a vulvovaginitis condition caused by non-*albicans* species selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae,* Appellants submit herewith a copy of a study published by Nyirjesy et al. on non-*albicans* Candida, and its role in vaginitis **subsequent to the filing date of the present application**. *See* Nyirjesy et al., "Vaginal *Candida parapsilosis:* Pathogen or bystander?" *Infectious Diseases in Obstertrics and Gynecology*, 13(1):47-41, March 2005 ("Exhibit 6"). According to the Nyirjesy et al. study, "C*andida parapsilosis* has been identified

as a vaginal isolate, but little evidence exists to support its role as a vaginal pathogen." *See* Nyirjesy et al. at p. 37, sentence bridging the first and second columns. Further, regarding *C. parapsilosis*, Nyirjesy et al. note "there have been no studies that look specifically at its relevance to symptoms." *See Id.* at p. 38, top of the first column. Finally, according to Nyirjesy et al., "**some investigators have questioned whether some non-*C. albicans* species cause vulvovaginal symptoms at all.**" *See Id.* at p. 39, in the first full paragraph in the second column. In view of Nyirjesy et al., it is clear that the state of the art at the time the present application was filed would not have considered the presently claimed subject matter.

Appellants submit that the scope and content of the prior art has been misconstrued. As evidenced by the express text of Lynch and Sobel, a need for further investigation on the role of butoconazole (and itraconazole) in experimental vaginitis was needed, especially with regard to vaginitis caused by non-*albicans* species. Additionally, as evidenced by the abstracts submitted herewith, Appellants note Sobel, even after Brown et al. was published, did not appear to obtain the presently claimed subject matter. Accordingly, Appellants submit that a person of ordinary skill in the art could only arrive at the presently pending subject matter by way of impermissible hindsight, i.e., construing the scope and contents of the prior art in view of the teachings of the present application.

b. All Elements Not Taught or Suggested

All of claims 1-35 are, generally, directed to the use of a bioadherant, single dose **treatment** of a vulvovaginitis condition **caused by** non-*albicans* species of *Candida*. Specifically, claims 1-35 are directed to treating conditions caused by *Candida* species including: *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

The '329 patent teaches systems, methods of preparation and administration of the release of an active agent in a controlled manner for an extended period in a vaginal cavity environment. The disclosure teaches that when the systems incorporate an antifungal agent, i.e., an imidazole, the conventional treatment time may be reduced by at least 25%. Specifically, the '329 patent teaches that the conventional treatment period or quantity of agent used is reduced by at least 25%, whereas normally a controlled release drug system reduces the number of times a day that a drug must be administered rather than the overall length of treatment. See, col. 4, lines 5-20. The '329 patent teaches that tests utilizing imidazoles upon *C. albicans* have demonstrated this result.

As discussed above, the presently claimed subject matter is the use of a **bioadherant**, **single dose** treatment of a vulvovaginitis condition caused by **non-*albicans*** species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*. The '329 patent does not teach or suggest the use of a bioadherent, single-dose treatment

of a vulvovaginatis condition caused by non-*albicans* as presently claimed. Therefore, the '329 patent does not teach each and every element of the presently pending claims as required by *In re Wilson*.

Brown et al. is discussed above with regard to the rejection under 35 U.S.C. § 102(b). Brown et al. is directed to a comparison of the safety and efficacy of (1) a single vaginal dose of butoconazole nitrate 2% sustained release cream with (2) a seven day schedule of miconazole nitrate 2% vaginal cream in the treatment of vulvovaginal candidias caused by *C. albicans*.

Appellants reiterate, the presently claimed subject matter is the use of a **bioadherant**, **single dose** treatment of a vulvovaginitis condition caused by **non-*albicans*** species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

Brown et al. does not remedy the deficiencies of the '329 patent. As discussed, Brown et al. does not teach or suggest the use of a bioadherent, single-dose treatment of a vulvovaginatis condition caused by non-*albicans* as presently claimed. Therefore, whether taken alone, or in combination, the '329 patent and Brown et al. do not render the presently claimed subject matter obvious.

Garg is merely directed to pharmaceutical excipients useful in vaginal formulations. Additionally Garg teaches the Regulatory Status to which some of the excipients disclosed therein are assigned.

As discussed, the presently claimed subject matter is the use of a **bioadherant**, **single dose** treatment of a vulvovaginitis condition caused by **non-*albicans*** species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

Garg does not remedy the deficiencies of the '329 patent and Brown et al. Garg does not teach or suggest the use of a bioadherent, single-dose treatment of a vulvovaginatis condition caused by non-*albicans* as presently claimed. Therefore, whether taken alone, or in combination, the '329 patent, Brown et al. and Garg do not render the presently claimed subject matter obvious.

Droegemueller et al. is directed to a three day treatment with butoconazole nitrate for vulvovaginal candidiasis. The teachings of Droegemuller et al. are limited to *C. albicans.*

Again, the presently claimed subject matter is the use of a **bioadherant**, **single dose** treatment of a vulvovaginitis condition caused by **non-*albicans*** species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

Droegemueller et al. does not remedy the deficiencies of the '329 patent, Brown et al. and Garg. Droegemueller et al. does not teach or suggest the use of a bioadherent, single-dose treatment of a vulvovaginatis condition caused by **non-*albicans*** species of *Candida* as presently claimed. Therefore, whether taken alone, or in combination, the '329 patent, Brown et al., Garg and Droegemuller et al. do not render the presently claimed subject matter obvious.

The '985 patent merely suggests a composition comprising an antifungal agent with polyglyceryl 2-4 oleate.

As stated, the presently claimed subject matter is the use of a **bioadherant**, **single dose** treatment of a vulvovaginitis condition caused by **non-*albicans*** species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

The '985 patent does not remedy the deficiencies of the '329 patent, Brown et al., Garg and Droegmueller et al.. The '985 patent does not teach or suggest the use of a bioadherent, single-dose treatment of a vulvovaginatis condition caused by non-*albicans* as presently claimed. Therefore, whether taken alone, or in combination, the '329 patent, Brown et al., Garg, Droegemueller et al. and the '985 patent do not render the presently claimed subject matter obvious a *prima facie case* of obviousness under 35 U.S.C. § 103(a) has not been established.

For these reasons, in addition to others not noted herein, Appellants submit a *prima facie* case obviousness has not been established. Accordingly, withdrawal of the subject rejection and allowance of claims 1-35 is respectfully requested.

CONCLUSION

For the foregoing reasons, Appellants respectfully submit that the Examiner's rejection of the presently pending claims was erroneous. Accordingly, Appellants respectfully request reversal of the Examiner's decision.

The Commissioner is authorized to charge Deposit Account No. 14-0112 for any additional charges in connection with this appeal.

The Examiner is welcomed to contact the undersigned attorney if such contact would be helpful in the further prosecution of this case.

Respectfully Submitted,

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Appendix A

Claims on Appeal

1. (Previously Presented) A method for the local treatment of a vulvovaginal candidiasis, which comprises:

treating said vulvovaginal candidiasis condition caused by a species of *Candida* selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae* by applying to the vaginal tissue of a human a formulation comprising:

about 35 to about 45% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1% w/w methylparaben; about 0.001 to about 1% w/w propylparaben; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate; and

wherein the treatment is a single dose treatment.

2. (Original) The method according to claim 1, wherein said formulation further comprises:

about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01 to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate.

3. (Original) The method according to claim 2, wherein said formulation further comprises:

about 39.978% w/w sorbitol solution; about 5% w/w propylene glycol; about 0.05% w/w edetate disodium; about 8.032% w/w mineral oil; about 2.713% w/w polyglyceryl-3-oleate; about 2.713% w/w glyceryl monoisostearate; about 0.452% w/w microcrystalline wax; about 1.013% w/w silicon dioxide; about 0.18% w/w methylparaben; about 0.05% w/w propylparaben; about 37.819% w/w water; and about 2.0% w/w butoconazole nitrate.

4. (Original) The method according to claim 3, wherein the species is *C. glabrata*.

5. (Original) The method according to claim 3, wherein the species is *C. tropicalis*.

6. (Original) A method for the treatment of a vaginal fungal condition, which comprises:

administering a single dose composition comprising about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01 to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate;

wherein the vaginal fungal condition is a vulvovaginal candidiasis condition caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*, and

wherein the ratio of polyglyveryl-3-oleate to glyceryl monoisostearate is about 1:0.1-10.

7. (Original) The method according to claim 6, wherein the species is *C. glabrata*.

8. (Original) The method according to claim 6, wherein the species is *C. tropicalis*.

9. (Original) A method for the treatment of an unidentified vulvovaginal fungal condition, which comprises:

administration to said fungal condition a bioadhesive, single dose treatment formulation comprising from about 0.500 to about 5.000% w/w butoconazole nitrate; and

wherein the unidentified vulvovaginal fungal condition is caused by a *Candida* species selected from the group consisting of *dubliniensis*, *tropicalis*, *glabrata*, *parapsilosis*, *krusei*, and *lusitaniae*.

10. (Original) The method according to claim 9, wherein said formulation further comprises: about 35 to about 45% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1% w/w methylparaben; about 0.001 to about 1% w/w propylparaben;

about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate.

11. (Original) The method according to claim 10, wherein said formulation further comprises:

about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01 to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate.

12. (Original) The method according to claim 10, wherein said formulation further comprises:

about 39.978% w/w sorbitol solution; about 5% w/w propylene glycol; about 0.05% w/w edetate disodium; about 8.032% w/w mineral oil; about 2.713% w/w polyglyceryl-3-oleate; about 2.713% w/w glyceryl monoisostearate; about 0.452% w/w microcrystalline wax; about 1.013% w/w silicon dioxide; about 0.18% w/w methylparaben; about 0.05% w/w propylparaben; about 37.819% w/w water; and about 2.0% w/w butoconazole nitrate.

13. (Original) The method according to claim 12, wherein the species is *C. glabrata*.

14. (Original) The method according to claim 12, wherein the species is *C. tropicalis*.

15. (Original) The method according to claim 10, wherein the bioadhesive formulation minimizes leakage from the vaginal cavity of a human.

16. (Original) The method according to claim 10, wherein the treatment provides peak plasma levels of the butoconazole nitrate at about 6 to about 48 hours after administration and retains activity for at least 4 days.

17. (Previously Presented) A method for the treatment of a fungal condition, which comprises:

applying to a vulvovaginal candidiasis condition caused by a member selected from the group consisting of *Candida dubliniensis*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, mycelial *Candida*, *Candida krusei*, and *Candida lusitaniae* and mixtures thereof of a treatment comprising:

about 35 to about 45% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1%

w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1% w/w methylparaben; about 0.001 to about 1% w/w propylparaben; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate.

18. (Original) The method according to claim 17, wherein the treatment is a single dose treatment.

19. (Previously Presented) A method for the local treatment of an unidentified vaginal fungal condition comprising:

administering a single administration of a composition consisting essentially of: about 38 to about 40% w/w sorbitol solution; about 4 to about 6% w/w propylene glycol; about 0.01 to about 0.5% w/w edetate disodium; about 6 to about 9% w/w mineral oil; about 2 to about 3% w/w polyglyceryl-3-oleate; about 2 to about 3% w/w glyceryl monoisostearate; about 0.01 to about 0.8% w/w microcrystalline wax; about 0.09 to about 0.9% w/w silicon dioxide; about 0.01 to about 0.5% w/w methylparaben; about 0.01 to about 0.5% w/w propylparaben; about 30 to about 40% w/w water; and about 1.5 to about 3.5% w/w butoconazole nitrate; and

wherein the administration is to a vulvovaginal candidiasis condition caused by any member selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae*.

20. (Original) The method according to claim 19, wherein the species is *C. glabrata*.

21. (Original) The method according to claim 19, wherein the species is *C. tropicalis*.

22. (Previously Presented) A method for the treatment of a fungal condition, comprising:

treating a candidiasis condition caused by a species selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* by applying to the vaginal tissue a multiphase formulation in a single dose;

wherein the multiphase formulation comprises:

a hydrophilic phase, which comprises: about 38 to about 40% w/w sorbitol solution; about 3 to about 8% w/w propylene glycol; about 0.001 to about 1% w/w edetate disodium; about 25 to about 45% w/w water; and about 0.5 to about 5% w/w butoconazole nitrate; and

a hydrophobic phase which comprises about 5 to about 11% w/w mineral oil; about 0.5 to about 5% w/w polyglyceryl-3-oleate; about 0.5 to about 5% w/w glyceryl monoisostearate; about 0.001 to about 1% w/w microcrystalline wax; about 0.5 to about 2% w/w silicon dioxide; about 0.001 to about 1.000% w/w methylparaben; and about 0.001 to about 1% w/w propylparaben.

23. (Original) The method according to claim 22, wherein the hydrophobic phase and hydrophilic stage for a bioadhesive dosage form provides peak plasma levels of butoconazole nitrate at about 6 to about 48 hours and retains activity for at least 4 days.

24. (Previously Presented) A method for the treatment of an unidentified vulvovaginatis condition comprising:

treating a condition caused by a species of *Candida* selected from the group consisting of *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* by applying to the vaginal tissue a multiphase formulation in a single dose to provide a *Candida* species kill rate of about 50 to about 100% for a period of at least about 4 days.

25. (Original) The method according to claim 24, wherein the multiphase formulation is administered via an applicator device which is designed to apply the formulation evenly over the vaginal tissue of a human.

26. (Original) A method according to claim 24, wherein the species is *C. glabrata*.

27. (Original) A method according to claim 24, wherein the species is *C. tropicalis*.

Appendix B

Evidence Appendix

In support of Appellants position outlined herein, submitted herewith please find copies of each of:

Exhibit 1. Lynch, ME, Sobel, JD: *Comparative in vitro activity of antimcotic agents against pathogenic vaginal yeast isolates*, J Med Vet Mycol (1994), 32:267-274.

Exhibit 2. Sobel, JD, "Treatment of vaginal Candida infections," *Expert Opin Pharmocother*, 3(8): 1059-65, Aug 2002. ABSTRACT

Exhibit 3. Moosa, MY and Sobel JD, "Non-*albicans* Candida infections in patients with hematologic malignancies," *Semin Respir Infect.*, 17, (2): 91-8, Jun 2002. ABSTRACT

Exhibit 4. Sobel et al., "Treatment of Complicated Candida vaginitis: comparison of single and sequential doses of fluconazole," *Am J Obstet Gynecol.*, 185(2): 363-9, Aug 2001. ABSTRACT

Exhibit 5. Sobel JD, "Antimicrobial Resistance in Vulvovaginitis," *Curr Infect Dis Rep*, 3(6): 546-549, Dec 2001. ABSTRACT

Exhibit 6. Nyirjesy et al., "Vaginal *Candida parapsilosis:* Pathogen or bystander?" *Infectious Diseases in Obstertrics and Gynecology*, 13(1):47-41, March 2005.

Exhibit 1 was submitted with an Information Disclosure Statement filed on July 30, 2004, along with an Information Disclosure Statement. The Examiner formally recognized, and indicated his consideration of, Exhibit 1 by initialing the PTO Form-1449 submitted on July 30, 2004. Accordingly, Appellants note that Exhibit 1 was formally recognized and entered into the record by the Examiner on May 18, 2006, subsequent to being filed on July 30, 2004.

Each of Exhibits 2-5 were submitted with Appellants' Response filed on August 9, 2007, along with an Information Disclosure Statement. The Examiner formally recognized, and indicated his consideration of, Exhibits 2-5 by initialing the PTO Form-1449 submitted on August 9, 2007. Accordingly, Appellants note that Exhibits 2-5 were formally recognized and entered into the record by the Examiner on August 23, 2007, subsequent to being filed on August 9, 2007.

Exhibit 6 was submitted with Appellants' Response filed on August 9, 2007. The filing receipt, stamped by the USPTO on August 9, 2007, serves as *prima facie* evidence that the Examiner received Exhibit 6.

Appendix C

Related Proceedings Appendix

NONE

Exhibit 5

1: Curr Infect Dis Rep. 2001 Dec;3(6):546-549.

Links

Antimicrobial Resistance in Vulvovaginitis.

Sobel JD.

Division of Infectious Diseases, Harper Hospital, 3990 John R, Detroit, MI 48201, USA. jsobel@intmed.wayne. edu

Although antimicrobial resistance has had an enormous impact on selection and utilization of antibiotics in virtually all aspects of clinical medicine, both inpatient and community based, little attention has been directed at antimicrobial resistance occurring in vaginal infections. Little evidence exists that frequent relapses of bacterial vaginosis or vulvovaginal candidiasis are due to antimicrobial resistance. Similarly, metronidazole-resistant trichomoniasis remains rare. Nevertheless, abuse of over-the-counter antimycotics, as well as widespread prescription of systemic oral azoles, could result in spread of azole-resistant Candida albicans, and even more likely could lead to an increase in non-albicans Candida species with intrinsic azole resistance. Problematic species include Candida glabrata and rarely Candida krusei.

PMID: 11722813 [PubMed - as supplied by publisher]

Infectious Diseases in Obstetrics and Gynecology, March 2005; 13(1): 37–41

CLINICAL STUDY

Vaginal *Candida parapsilosis*: Pathogen or bystander?

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Abstract

***Objective*:** *Candida parapsilosis* is an infrequent isolate on vaginal cultures; its role as a vaginal pathogen remains unstudied. This retrospective study of women with positive culture for *C. parapsilosis* sought to characterize the significance of this finding and its response to antifungal therapy.

***Methods*:** From February 2001 to August 2002, we identified all individuals with positive fungal isolates among a population of women with chronic vulvovaginal symptoms. Charts of women with *C. parapsilosis* cultures were reviewed with regard to patient demographics, clinical presentation and therapeutic response. Mycological cure, defined as a negative fungal culture at the next office visit, and clinical cure, i.e. symptom resolution, were determined for each subject.

***Results*:** A total of 582 women had positive vaginal cultures for 635 isolates, of which 54 (8.5%) were *C. parapsilosis*. The charts of 51 subjects with *C. parapsilosis* were available for review and follow-up cultures and clinical information were available for 39 (76.5%). Microscopy was positive in 9 (17.6%). Antifungal treatment resulted in mycological cure in 17/19 patients with fluconazole, 7/7 with butoconazole, 6/6 with boric acid, 1/1 with miconazole and occurred spontaneously in 6/7: 24/37 (64.9%) patients with a mycological cure experienced clinical cure.

***Conclusions*:** Although *C. parapsilosis* is often a cause of vaginal symptoms, it seems to respond to a variety of antifungal agents and may even be a transient vaginal colonizer.

Keywords: *Vaginitis, vulvovaginal candidiasis, Candida parapsilosis*

Introduction

Vaginitis is the most common reason for patient visits to obstetrician-gynecologists and accounts for over 10 million physician office visits annually [1]. Among the most common diagnosis in women presenting with vaginal irritation is vulvovaginal candidiasis(VVC); 80% to 90% of sporadic, uncomplicated cases of VVC are caused by the species *Candida albicans* [2]. However, other species may be responsible for up to 30% of recurrent VVC cases [3]. The identification of non-*C. albicans* species in vulvovaginal infection is important because some non-*C. albicans* species are resistant to the standard azole therapy used to clear the infection. The most common non-*C. albicans* species that have been implicated in recurrent VVC include *Candida glabrata, Candida tropicalis, Candida krusei,* and *Saccharomyces cerevisiae*. To a lesser extent *Candida parapsilosis* has been identified as a vaginal isolate, but little evidence exists to support its role as a vaginal pathogen; it may simply represent colonization of the normal vaginal environment.

The identification of non-*C. albicans* species on vaginal fungal culture has become more common in recent years. This may partially be due to the increased usage of vaginal fungal cultures for accurate diagnosis of complicated or recurrent VVC, as recommended by several authors [3, 4]. Others believe that the increase in non-*C. albicans* isolates is secondary to the increased use and availability of over-the-counter antimycotic preparations [5, 6]. Regardless of the reason, a positive culture for non-*C. albicans* yeast species such as *C. parapsilosis* from a symptomatic patient may sometimes lead to treatment. However, with the less common types of yeast, determining whether treatment is appropriate and what it should consist of may not be clear.

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Poster presentation at the 2003 Annual Meeting of the Infectious Diseases Society for Obstetrics and Gynecology, Hyannis, Massachusetts, USA.

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C. parapsilosis is a relatively infrequent isolate on vaginal fungal culture, and there have been no studies that look specifically at its relevance to symptoms. Because *C. parapsilosis* produces certain virulence factors such as acid proteinases, it has been hypothesized that this organism is a vaginal pathogen [7] but it remains relatively unstudied as a cause of VVC. The purpose of this descriptive study was to determine the prevalence of *C. parapsilosis* isolates in our population, to evaluate the symptoms experienced by women with positive vaginal cultures, to examine the effectiveness of different antifungal remedies against *C. parapsilosis* and to determine whether a negative follow-up culture was associated with the relief of vaginal complaints. In doing so, our goal was to determine whether *C. parapsilosis* represents a true vaginal pathogen.

Methods

The study population was derived from women referred by their primary care physicians for medical treatment of chronic vulvovaginal complaints at an outpatient vaginitis referral center. From February 2001 to August 2002, all individuals with positive vaginal fungal isolates were identified using office flowsheets maintained to track and notify patients of their results. All women with a positive culture for *C. parapsilosis* were selected for retrospective chart review. Data regarding demographics, medical history, symptom history, and treatment were obtained from standardized patient chart notes. Follow-up information was obtained from additional chart entries. The Institutional Review Board at Thomas Jefferson University Hospital approved the study protocol.

Two clinicians, both specialized in the treatment of chronic vulvovaginal complaints, collected data and examined all subjects throughout the study period. Saline wet-mount preparations, 10% potassium hydroxide (KOH) preparations, and vaginal pH determinations were obtained routinely for women seen at the center. Saline and KOH preparations were performed by spreading vaginal secretion samples on separate slides, adding the appropriate solutions, and then evaluating with both low- and high-power microscopy for the presence of fungal elements, altered vaginal flora, clue cells, trichomonads, vaginal cytology and white blood cells. Sterile culture swabs were used to sample the external vulvar skin and lateral vaginal sidewalls on speculum exam for yeast cultures. Three laboratories analyzed vaginal swabs for fungal isolates. The patient's insurance carrier dictated which laboratory site was used to analyze the fungal swab.

The Thomas Jefferson University laboratory (Philadelphia, PA, USA) identified yeast isolates by first plating vaginal swabs onto CHROMagar plates. If growth was seen on the CHROMagar, then a saline wet mount was prepared to confirm the presence of yeast species. Germ tube-positive species were identified as *C. albicans*. Germ tube-negative colonies were further speciated using the Rapid ID System (Remel, Lenexa, KS, USA). This presumptive diagnosis was simultaneously confirmed using cornmeal agar and urea tubes. Quest Diagnostics Laboratory (Philadelphia, PA, USA) used inhibitory mold agar for the initial plating of vaginal swabs. Germ tube-negative colonies were further isolated using Sabouraud agar. Pure colonies were then speciated using API 20 C (BioMerieux Vitek Inc., Hazelwood, MO, USA), a carbohydrate assimilation test, in conjunction with morphology testing for proper identification. Laboratory Corporation of America (New Castle, DE, USA) initially seeded Sabouraud-dextrose agar and Mycosel agar with vaginal swabs. Germ tube-positive species were confirmed as *C. albicans* by the concurrent formation of chlamydospores in cornmeal agar. Germ tube-negative colonies were further speciated using the YBC card (BioMerieux Vitek, Hazelwood, MO, USA). Confirmatory testing was performed using the API 20 C system.

Symptoms were documented at the index visit and at the follow-up visit. Follow-up visits occurred between 1 and 4 months after the initial *C. parapsilosis* culture. Intervening treatments and compliance with treatment were reviewed, as well as change in vaginal symptoms. A clinical cure was defined as complete resolution of the symptoms noted at the time of the index visit. Mycological cure was defined as resolution of *C. parapsilosis* on follow-up culture. Cases were documented as a spontaneous mycological cure if the follow-up culture did not grow *C. parapsilosis* and antifungal treatment was never initiated.

Statistical analysis was performed using EpiInfo 2002 (CDC, Atlanta, GA, USA). Two- tailed chi-square statistical analysis was carried out using the Mantel—Haenszel formula. Statistical significance was defined as a p value < 0.05.

Results

A total of 582 women had positive vaginal cultures for 635 isolates; 609 organisms were grown on pure culture and 13 cultures contained growth of mixed species. Table I shows the distribution of isolates among this patient population. Isolates positive for *C. parapsilosis* were found in 54 (8.5%) of women, 1 of whom had a culture positive for both *C. albicans* and *C. parapsilosis*. The charts of 51 patients with *C. parapsilosis* were available for review, and follow-up culture and clinical information was available in 39

Table I. Distribution of yeast isolates.

Species	Number	Percentage
Candida albicans	457	72.0%
Candida glabrata	74	11.7%
Candida parapsilosis	54	8.5%
Rhodotorula spp.	18	2.8%
Saccharomyces cerevisiae	9	1.4%
Candida lusitaniae	5	0.8%
Other species	18	2.8%

(76.5%). With the exception of 3 subjects, all women were seen for follow-up within 6 weeks of the index visit.

The median patient age was 46 years (range 19 to 86 years); 49 women (96.1%) were Caucasian and 18 (35.3%) were nulliparous. Of the 21 women (41.2%) who were menopausal, 19 (90.5%) were receiving estrogen therapy. Oral contraceptives were being used by11 (21.6%), and 21 (41.2%) had used antifungals and 10 (19.6%) had used topical steroids within 1 month of positive culture. At the time of the index visit, complaints comprised itching in 27 (53%), burning in 22 (43.1%), abnormal discharge in 11 (21.6%) and dyspareunia in 16 (31.4%) women. However, 9 (17.6%) were asymptomatic at the time of positive culture although microscopy was also positive, and of these 4 were seen for a follow-up visit.

In this study, 37 women (72.5%) had associated vulvovaginal conditions. Of these, the most common conditions were atrophic vaginitis in 11 (29.7%), irritant dermatitis in 8 (21.6%), lichen sclerosus in 8 (21.6%) and vulvar vestibulitis in 5 (13.5%). Other diagnoses included vulvodynia, herpes simplex, recurrent bacterial vaginosis and desquamative inflammatory vaginitis, and 8 women (21.6%) carried the diagnosis of two vulvovaginal conditions in addition to *C. parapsilosis* vaginitis. Between the index and follow-up visits, the only change in treatment was the institution of antifungal therapy.

A variety of antifungal regimens were used in patients with cultures positive for *C. parapsilosis*. The treatments included boric acid, 600 mg twice daily for 2 weeks, buconazole, two vaginal applicator doses 1 week apart, fluconazole, 200 mg twice weekly for 1 month, and miconazole, one applicator nightly for 7 days. The choice of antifungal agent was left to the discrimination of the clinician. Antifungal treatment resulted in mycological cure in 17/19 cases with fluconazole, 7/7 with buconazole, 6/6 with boric acid, and 1/1 with miconazole. Mycological cure also occurred spontaneously in 6/7 women, of whom 24/37 (64.9%) experienced clinical cure. Of those with associated vulvovaginal conditions, 14/26 women achieved both mycological and a clinical cures (10 with treatment and 4 without treatment), whereas 10/13 without associated vulvovaginal conditions achieved both clinical and mycological cures (9 with treatment and 1 without treatment).

Discussion

More than 80% of VVC cases are caused by the species *C. albicans*. In mild cases the organism responds to a variety of standard azole remedies, wheeas complicated or recurrent cases respond to more aggressive multiple-dose regimens. The remaining cases of vaginal candidasis are caused by non-*C. albicans* species that appear to have higher minimum inhibitory concentrations to standard azole therapies [6]. Additionally, some investigators have questioned whether some non-*C. albicans* species cause vulvovaginal symptoms at all [7, 8]. Most of these studies evaluated the non-*C. albicans* isolates collectively, without studying symptomatology or the mycotic response of minor isolates individually. To our knowledge, this is the largest study that looks exclusively at the minor isolate *Candida parapsilosis*, its prevalence, symptomatology and mycotic response.

The prevalence of *Candida parapsilosis* in our study was slightly higher (8.5%) than that previously documented. Other authors report prevalence of 5% or less for *C. parapsilosis* in symptomatic patients; however, these studies evaluate prevalence in a much smaller population than ours [3, 4]. Sood et al. report a prevalence of 12% in their study of terconazole for treatment of non-*C. albicans* vaginitis, but studied only 28 patients, 3 of whom had *C. parapsilosis* isolates [9]. Because this study was specifically a study of non-*C. albicans* cases, the 12% incidence of *C. parapsilosis* in the series may not be an accurate reflection of the incidence in larger population of women with complicated VVC. Our study may over-represent the true prevalence because of the selection bias of our population. Women seeking treatment at a vaginitis referral center may be more likely to have cultures positive for *C. parapsilosis*, because cases of uncomplicated candidiasis are eliminated from a referred population.

The symptoms experienced by women with *C. parapsilosis* infection were typical of any vulvovaginal infection. Complaints included itching (53%), burning (43.1%), dyspareunia (31.4%) and abnormal discharge (21.6%). Approximately 20% appeared to be asymptomatically colonized with *C. parapsilosis* at the index visit. Certainly, the reported complaints are not unique to VVC and could also be attributed to secondary diagnoses affecting the vulvovaginal area, which were present in 72.5% of the study population. Likewise, objective findings suggestive of candidiasis

were unhelpful in diagnosing vaginal *C. parapsilosis*, as only 17.6% of cases demonstrated yeast species on saline microscopy. In the few subjects with positive microscopy, the objective finding was not helpful in predicting whether the woman would achieve a mycotic and symptomatic cure with treatment. This underscores the usefulness of vaginal fungal cultures for deciphering the diagnostic ambiguity of vulvovaginal conditions. Without a positive fungal culture, the isolate could masquerade as a number of other conditions, eluding appropriate treatment.

When pretreatment and post-treatment symptoms were compared, the data from this study strongly suggested that vaginal *C. parapsilosis* can be a pathogen responsible for vulvovaginal complaints. Symptomatic relief was experienced by 65% of women who cleared the isolate on follow-up culture. In those who did not report symptomatic relief at their follow-up visits, 10/13 had other vulvovaginal conditions. It is possible that *C. parapsilosis* was contributing to their symptoms but that their other problems prevented complete symptomatic relief. Alternatively, it may be that *C. parapsilosis* was an innocent bystander in those cases where clearance was not associated with clinical cure. Furthermore, in women with other vulvovaginal conditions who did get better, it is possible that their improvement was not secondary to the disappearance of *C. parapsilosis*, but rather to improvement of their other conditions with further time.

Agatensi and colleagues hypothesized that *C. parapsilosis* is a potential vaginal pathogen, in that isolates demonstrate acid (aspartyl) proteinase activity [7]. This enzyme is capable of hydrolyzing mucosal IgA and interfering with the natural vaginal barrier to infection. Additionally, the isolates cultured from symptomatic subjects demonstrated significantly higher proteinase activity than control cultures. The only other candidal isolate capable of significant acid proteinase activity is *C. albicans*, a known vaginal pathogen. It seems logical that the proteolytic acitivity shared by both *C. parapsilosis* and *C. albicans* may explain their common behavior as pathogenic organisms. Additionally, women suffering with multiple vulvovaginal diagnoses, and with theoretically compromised integrity of the vaginal mucosa, may be more susceptible to infection with *C. parapsilosis* because of the acid proteinase activity of the organism. This suggestion is supported by the observation that several of the women who spontaneously cleared *C. parapsilosis* did so while receiving non-antimycotic treatment for other vulvovaginal conditions. Perhaps restoration of healthy vaginal epithelium diminishes the ability of *C. parapsilosis* to infect its host.

Despite its apparent virulent capability in the vagina, our data also suggest that vaginal infection with *C. parapsilosis* is treated and cleared from subsequent culture relatively easily. In all but 2 cases in this series, the infection cleared with a single course of antimycotic therapy. Ot these 2 cases, 1 cleared the isolate with a second antimycotic agent, and the other cleared the isolate while receiving steroid treatment for a separate vulvovaginal condition. Admittedly, there is an inherent treatment bias that may skew the results toward a relatively high mycotic response rate, in that all the women received fairly aggressive treatment regimens. The finding that *C. parapsilosis* seems to clear fairly easily with antifungal therapy is not too surprising. We did not obtain *in vitro* susceptibility testing of our isolates to various antifungal agents. However, when Lynch and Sobel evaluated 377 clinical vaginal yeast isolates, they found that the 26 *C. parapsilosis* isolates seemed to have sensitivities which were quite similar to those of the *C. albicans* isolates [10]. Interestingly, 6 subjects cleared the isolate without specific antifungal therapy.

Further study of *Candida parapsilosis* should prospectively compare mycotic response with standard single-dose azole treatment, aggressive multiple dose regimens, and no treatment. In comparing these treatment groups, it may become clear that *C. parapsilosis* does not demonstrate the inherent azole resistance displayed by other non-*C. albicans* species. The number of cases of spontaneous isolate resolution suggests that *C. parapsilosis* may have limited virulent longevity in the vaginal environment.

In summary, *Candida parapsilosis* is a significant non-*C. albicans* vaginal isolate responsible for vulvovaginal complaints. Even when it appears to be a transient vaginal colonizer, it may be associated with vulvovaginal symptoms. In symptomatic patients, antifungal treatment should be expected to achieve symptomatic cure in a large number of patients. Properly controlled studies are still necessary to determine the most efficient antimycotic treatment regimen. In view of the relative rarity of this organism, an appropriately powered, randomized controlled trial is unlikely. However, in cases with a complicated history of recurrent candidiasis, extended antifungal treatment with fluconazole, buconazole, miconazole, or boric acid is reasonable but may be more aggressive than truly necessary.

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Exhibit 1

Butoconazole Nitrate 2% for Vulvovaginal Candidiasis

New, Single-Dose Vaginal Cream Formulation vs. Seven-Day Treatment with Miconazole Nitrate

Dale Brown, M.D., Milan R. Henzl, M.D., Raymond H. Kaufman, M.D., and the Gynazole 1 Study Group

OBJECTIVE: To compare the safety and efficacy of a single vaginal dose of a butoconazole nitrate 2% bioadhesive, sustained-release cream (butoconazole 1-BSR) with a seven-day schedule of miconazole nitrate vaginal cream 2% (miconazole 7).*

STUDY DESIGN: The clinical trial was conducted according to a randomized, parallel, investigator-blind, multicenter study design. The patients self-administered the respective creams to the posterior vaginal fornix. Two hundred twenty-three patients started the trial and were analyzed for safety. A total of 205 patients qualified for efficacy analysis, 101 receiving butoconazole 1-BSR and 104 using miconazole 7. Patients receiving butoconazole 1-BSR inserted one applicator full of medication once. Those assigned to receive miconazole 7 inserted one applicator full daily for seven days. Patients were evaluated 7–10 and 30 days after completion of therapy.

This clinical trial established the therapeutic equivalency of butoconazole 1-BSR and miconazole 7.

RESULTS: Butoconazole 1-BSR rapidly relieved the signs and symptoms of vulvovaginal candidiasis. The proportion of patients with severe symptoms declined from the pretreatment 20% to 6% on the 1st day, to 3% on the 4th day, and to 2–1% on the 5th–7th day after single-dose application. Eight to ten days after treatment completion, clinical symptoms regressed in 92%, and fungal cultures were negative in 87% of patients. At the 30-day posttreatment visit, 88% of patients remained clinically cured, and 74% had negative fungal cultures. In the miconazole 7 group, the proportion of patients with severe symptoms declined from 23% to 19% after

*Gynazole™ 1 (KV Pharmaceutical Co., St. Louis, Missouri) is a single-dose vaginal application of butoconazole nitrate 2% in a bioadhesive, sustained-release formulation.

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the first dose; thereafter, symptom relief proceeded more rapidly. Eight to ten days after treatment completion, clinical symptoms regressed in 92% and fungal cultures were negative in 87% of patients. At the 30-day follow-up examination, 86% patients were clinically cured, and 77% were culture negative. After single-dose butoconazole 1-BSR, severe symptoms receded faster than after the first dose of miconazole 7, and the difference was statistically significant (P = .01). In all other efficacy parameters, the differences between the two groups were not statistically significant. Neither treatment regimen caused significant adverse events.

CONCLUSION: This clinical trial demonstrated that butoconazole 1-BSR is an effective and safe alternative to longer-term therapy with miconazole nitrate (seven days) for vulvovaginal candidiasis. (J Reprod Med 1999;44:933–938)

Keywords: candidiasis, vulvovaginal; miconazole; butoconazole.

Introduction

Vulvovaginal candidiasis is one of the most frequently encountered vaginal infections in the United States, accounting for 13 million cases yearly.[1] After the highly active antifungal imidazoles were introduced for therapy for vulvovaginal candidiasis, about 20 years ago, two important developments took place: one extended our knowledge of the pathogenesis of the disease, and the other changed our view of the required duration of effective medical treatment.

Of the about 150 known species of *Candida*, only 9 are pathogenic in humans. Of these, *Candida albicans* is responsible for the development of vulvovaginal candidiasis in 80–92% of cases[1,2]; this species has been primarily targeted in the development of the imidazole treatment regimens. The eight non-*albicans* species include *glabrata, tropicalis, pseudotropicalis, lusitaniae, crusei, rugosa, parapsilosis* and *guilliermondi*. (*Candida stellatoidea* is now included among *C albicans*.) Although *C albicans* remains the prevalent noxious agent in vulvovaginal candidiasis, an increase in non-*albicans Candida* species has been reported, from 10% to 20%, during the last 20 years, and this is of concern.[1] *C glabrata* is the second most frequently encountered species, and it is considered to be less susceptible to standard treatment.[1-3]

Imidazoles remain the preferred first-line therapy for vulvovaginal candidiasis. However, it is recognized that up to 50% of patients stop treatment after they experience relief of symptoms.[4] In order to increase compliance, a trend has evolved toward shortening the time of drug application. The treatment courses of miconazole nitrate and clotrimazole have been gradually shortened, from the original 14 days to 7 and 3 days. Butoconazole nitrate vaginal cream 2% has been found highly effective in the three-day treatment schedule.[5]

Ultimately, clinical researchers aimed at the development of single-dose treatment. For a period of time, clotrimazole was used in a single-dose vaginal tablet. Currently, a vaginal ointment containing the imidazole tioconazole is available for single-dose application (Vagistat 1®, Bristol-Myers-Squibb, Princeton, New Jersey). Tioconazole is also the active ingredient in Monistat 1® (Ortho Pharmaceutical Corporation, Raritan, New Jersey), a trade name that has been in use for the three- and seven-day treatments with miconazole nitrate. While the concentrations of the imidazoles in creams for the three- and seven-day schedules are 1% (clotrimazole) and 2% (miconazole and butoconazole nitrates), the single-dose ointment contains tioconazole in a 6.5% concentration, more than three times higher.

In order to achieve single-dose treatment without increasing the concentration of the active imidazole or compromising efficacy, a new bioadhesive, sustained-release cream vehicle was designed for the vaginal administration of butoconazole nitrate 2% (butoconazole 1-BSR).

Butoconazole nitrate was chosen for its highly acceptable safety profile and proven clinical efficacy when applied as a "conventional" vaginal cream.[5-7] Furthermore, carefully conducted tests demonstrated its broad antifungal spectrum, consistently showing high activity against the most important eight non-*albicans Candida* species. Butoconazole 1-BSR was found to be superior to the currently used imidazoles—miconazole, clotrimazole, ketoconazole and terconazole—in inhibiting the growth of *C albicans* as well as the non-*albicans* pathogenic species.[8] Assays established the minimum inhibitory concentration of the tested imidazoles, the minimum concentration that inhibits the growth of the various *Candida* species by 80%.[8] Although *in vitro* susceptibility cannot always be extrapolated into clinical results, *in vitro* tests predicted clinical failure of fluconazole and terconazole with respect to *C glabrata* and *Saccharomyces cerevisiae*.[3,9]

This article presents data demonstrating that a single vaginal dose of butoconazole 1-BSR is clini-

cally equivalent to the conventional vaginal cream with miconazole nitrate 2% given for seven consecutive days (miconazole 7).

Methods

Study Design

The clinical trial was conducted according to a randomized, parallel-group, investigator-blind, multicenter study design. All participating patients signed an informed consent form, and those enrolled in California also read and signed the State of California Experimental Subject's Bill of Rights. The respective institutional review boards of the participating centers approved the clinical trial protocol.

Study Drugs and Their Assignment

According to a computer-generated table of random numbers, individual patients received identical boxes with the study medications. A person other than the investigator distributed the boxes. Patients assigned to a single dose of butoconazole nitrate received a filled applicator containing 5 g of butoconazole 1-BSR cream 2%. Patients assigned to miconazole 7 received a 45-g tube of miconazole nitrate vaginal cream 2% and a reusable vaginal applicator designed to deliver 5 g of the cream, for a total of seven applications. The study coordinator at each investigative center instructed the patients not to reveal the type of medication they received and the number of applications to the investigator.

Since butoconazole 1-BSR is a new addition to the short-term management of vulvovaginal candidiasis, its properties are described below in detail.

The bioadhesive, sustained-release (BSR) cream containing butoconazole 2% differs from the currently available "conventional" cream preparations in several respects. The "continuous" phase of the cream comes in contact with biologic surfaces and lowers their surface tension. This characteristic provides conditions for interfacial adhesion between the cream and the surface of the vaginal lining and permits spread of the cream over vaginal mucosal surfaces. *In vitro* studies have shown that in an acetate buffer of pH 4.3, which simulates normal vaginal fluid, BSR cream is highly stable and remains intact for a prolonged period of time. Butoconazole nitrate is released from the BSR cream in a sustained fashion, continuously, over a period of seven days. Under the same conditions, the conventional cream rapidly disintegrates, and butoconazole nitrate is released into the medium within four hours.[10]

Clinical data have demonstrated that butoconazole nitrate 2% formulated as BSR cream is present in the vagina 63% longer than butoconazole nitrate 2% administered in the conventional formulation. The median vaginal retention time for butoconazole 1-BSR was 4.20 days, while for the conventional formulation it was 2.57 ($P = .0024$).[11]

In patients with active vulvovaginal candidiasis, systemic absorption of butoconazole nitrate 2% from BSR cream was 1.7%, while from the conventional formulation it was higher, 5.5%.[5,11]

Patient Selection

Healthy, nonpregnant women with clinical signs and symptoms of vulvovaginal candidiasis participated in the study. KOH vaginal smears and samples of vaginal fluid were cultured on Sabouraud's and Micosel media (BBL Microbiology Systems, Cockeysville, Maryland) and confirmed or excluded the clinical impression of vulvovaginal candidiasis. Two microbiologic laboratories identified blindly the presence of *C albicans*. Examination of wet smears showing trichomonads or clue cells associated with bacterial agents excluded patients from enrollment. A test for chorionic gonadotropin was performed prior to admission to the study.

Examinations and Assessment

At admission and at each follow-up visit, fungal cultures were taken and vulvovaginal examinations performed, including a KOH smear. The window for the first follow-up visit was 7–10 days after therapy; for the second follow-up visit it was approximately 30 days after completion of treatment. Patients with negative initial fungal cultures were withdrawn from the study.

At each examination, the investigator focused on objective signs of vulvovaginal inflammatory changes, such as redness, edema, fissures, excoriations, ulcerations and discharge, and recorded subjective vulvovaginal symptoms, such as burning and itching. The investigator graded the intensity of signs and symptoms as 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The investigator also inquired about adverse events and concomitant medications.

The patients kept a daily record of symptoms of itching, rash, burning and discharge starting before drug application (day 0) and continuing for seven days. Patients receiving miconazole 7 rated the symptoms on each of the seven days shortly before they applied the drug. Patients graded each symptom as 0 = none, 1 = mild, 2 = moderate, and 3 = se-

vere. The scores for the individual symptoms of each patient were added to give a total severity score. For each treatment group, the sum of total scores for individual patients was calculated. This enabled daily comparison of symptom relief or lack of it between treatments during the first week of therapy.

Absence of *C albicans* in fungal cultures constituted the microbiologic cure. Remission of symptoms and signs of vulvovaginal candidiasis constituted the clinical cure. At the first follow-up visit, at least 50% of the entrance signs and symptoms of vulvovaginitis had to be absent, and the remaining ones had to improve from higher severity degrees to milder ones. Further improvement had to occur by the second follow-up visit.

Statistical Analysis

Admission data were analyzed using either the nonparametric Kruskal-Wallis procedure[12] or parametric analysis of variance.[13] The differences in cure rates between the two treatments were analyzed using the χ^2 test and Fisher's exact test (two tailed).[14] The differences between the compared parameters of efficacy were considered statistically significant at the level of .05. The lower limits of the one-sided 95% confidence intervals on the differences in cure rates between butoconazole 1-BSR and miconazole 7 were calculated and used to establish clinical equivalence of the two treatments. Clinical equivalence was declared when the lower limit of the 95% one-sided confidence intervals of the cure rate differences between butoconazole 1-BSR and miconazole 7 did not exceed 20. The sample sizes of the two compared treatment groups were sufficiently large to detect a 20% difference with 80% power.

Results

Demographics and Disease History

Two hundred twenty-three patients started the study and received the study medication. They all were included in the analysis of safety. Two hundred five patients were analyzed for efficacy. There were no statistically significant differences in demographic parameters between the two treatment groups. The patients were comparable with respect to age and gravidity/parity. Approximately two-thirds of the patients developed their first episode of vulvovaginal candidiasis at a mean age of 28 (±8), years and one-third of the patients had experienced one or more prior episodes of vulvovaginal candidiasis. About 50% of patients were gravida 0, para 0. The mean duration of the current episode of vulvovaginal candidiasis was 14 days for the butoconazole 1-BSR group and 10 days for the miconazole 7 group, with a large SD on the mean (50 and 20 days for the two respective groups), indicating the wide variability of this parameter.

Efficacy

The efficacy analyses were conducted on 109 patients receiving butoconazole 1-BSR and 114 patients receiving miconazole 7. The rapidity of relief of symptoms is illustrated in Figure 1, and the clinical and microbiologic cure rates are summarized in Table I.

Relief of Signs and Symptoms of Vulvovaginal Candidiasis, Assessed from Patient Diaries. Patients in the butoconazole 1-BSR treatment group achieved rapid relief of the signs and symptoms of vulvovaginal candidiasis, as illustrated in Figure 1. Of patients receiving butoconazole 1-BSR, 20% had severe symptoms before using medication. The proportion of patients with severe symptoms declined to 6% on the first day and to 3% on the fourth day after the single application of medication. On days five to seven, the proportion of patients with severe symptoms declined further, to 2% and 1%. In

Table I *Efficacy of Butoconazole 1-BSR, Single-Dose Application, vs. Miconazole, Seven-Day Treatment*

	Treatment groups		
Cure rate	Butoconazole 1-BSR cream, single dose	Miconazole cream 2%, 7-day treatment	P*
Clinical			
1st Follow-up: no. of patients treated/cured (%)	101/93 (92)	104/100 (96)	.24
2nd Follow-up: no. of patients treated/cured (%)	84/74 (88)	93/80 (86)	.92
Microbiologic			
1st Follow-up: no. of patients treated/cured (%)	98/85 (87)	101/93 (92)	.75
2nd Follow-up: no. of patients treated/cured (%)	77/57 (74)	87/67 (77)	.24

*P value of treatment differences between the two groups (see details in text).

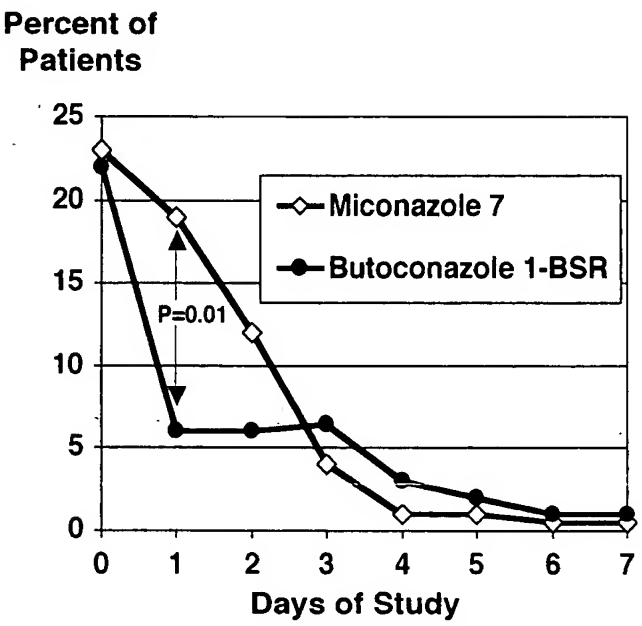

Figure 1 Proportion of patients with severe symptoms of vulvovaginal candidiasis after single application of butoconazole 1-BSR and during seven daily applications of miconazole vaginal cream 2%.

the miconazole 7 group, the proportion of patients with severe symptoms declined from 23% to 19% after the first dose; thereafter, symptomatic relief proceeded more rapidly. The difference between the rapidity of relief of severe symptoms after the two treatment schedules was statistically significant in favor of butoconazole 1-BSR ($P = .01$).

Clinical Cure Rate. Both tested compounds achieved high clinical cure rates at the first follow-up examination: butoconazole 1-BSR 92% and miconazole 7 96%. The 4% difference between treatment in favor of miconazole 7 was statistically nonsignificant.

Thirty days after treatment completion, the clinical cure rate for butoconazole 1-BSR was 88%, 4% lower than at the first follow-up examination. For miconazole 7, the cure rate was 86%, 10% lower than at the first follow-up examination. The 2% difference between treatments in favor of butoconazole was statistically nonsignificant.

Microbiologic Cure Rate. At the first follow-up visit, the microbiologic cure rates effected by butoconazole 1-BSR and miconazole 7 were 87% and 92%, respectively. Of the patients who were culture negative at the first follow-up examination and proceeded to the 30-day posttreatment examination, 74% treated with butoconazole 1-BSR were free of *C albicans*. In the miconazole 7 treatment group, the cure rate was 77%. The differences in microbiologic cure rates between the two treatments were statistically nonsignificant.

The lower limit of the 95% confidence interval on all efficacy differences between the two compared treatment schedules was <20%; therefore, the two treatment regimens are clinically equivalent.

Safety

No systemic adverse events occurred. Three patients receiving butoconazole 1-BSR and two receiving miconazole 7 complained of increased vulvovaginal irritation (burning and itching). One patient in each of the respective treatment groups dropped out of the study because of these symptoms.

Discussion

This clinical trial demonstrated that for vulvovaginal candidiasis, butoconazole 1-BSR is an effective and safe alternative to longer-term treatment schedules, such as the conventional seven-day application of miconazole nitrate cream 2%, used in this study. One drawback in the design of the study was the different duration of therapy used by the two treatment groups. We had to depend upon the patients to maintain confidentiality about their treatment group when examined by the evaluating physician.

Effective single-dose treatments with potent antifungal imidazoles appear to be almost ideal for the clinical management of vaginal fungal infections. For the physician, they virtually guarantee full compliance. Single-dose therapy reduces the inconveniences of longer-term schedules to a minimum. Repeated applications are especially unpleasant for the physically active or frequently travelling woman. Further inconveniences are leakage and possible restriction of sexual life during repeated applications.

In the development of single-dose treatments, the challenge was to maintain the high degree of efficacy and safety that has been associated with longer-term schedules. In the two single-dose preparations available so far, this problem was approached by an increase in the imidazole concentration. In the clotrimazole single-dose vaginal insert, the dose

was elevated to 500 mg, while the tablets used in the three- and seven-day schedules contain only 100 mg of imidazole. The high drug content necessitated augmentation of the insert size; some patients perceived that as inconvenient. Some patients also experienced problems with *in situ* dissolution of the insert. In the single-dose ointment, tioconazole was concentrated to 6.5%, a substantial increment over the 1% and 2% drug concentrations in the longer-term antifungal vaginal creams. The impact of the rapidly released higher amount of active imidazole on the vaginal mucosa could lead to increased occurrence of irritative symptoms (see below).

In the development of butoconazole 1-BSR, the researchers aimed at maintaining the low (2%) concentration of butoconazole. They focused on formulating a vaginal cream that would adhere to the vaginal mucosal surface for a prolonged period of time and would release the active ingredient in a continuous manner. Such a formulation would provide sustained contact of imidazole with the noxious agent and secure high antifungal activity without unnecessary leakage and symptoms of vaginal irritation.

Clinical studies and experience confirmed these assumptions.[5,11] The sustained-release formulation is retained in the vagina about twice as long as the conventional vaginal cream, and systemic absorption is three times lower from the BSR formulation than from the conventional cream. As shown by this study, a single vaginal application of butoconazole 2% in BSR cream provided cure rates comparable to those of the well-established seven-day treatment regimen of daily applications of miconazole nitrate 2% in the conventional vaginal cream. Although patients assigned to miconazole 7 used the cream for a full week, statistical analysis of the therapeutic differences between butoconazole 1-BSR and miconazole 7 demonstrated that the two treatments are equivalent. An added advantage of butoconazole 1-BSR is the significantly faster relief of severe symptoms of vulvovaginal candidiasis, on the first posttreatment day.

With respect to safety, only indirect comparisons are available. A clinical monograph on one-dose tioconazole vaginal ointment 6.5% reports local burning and itching in 6% and 4.8% of patients, respectively, and vulvar and vaginal irritation symptoms in 1.6%. Vaginal leakage was reported by 29% of patients; however, only 1% found it unacceptable.[15] Registration studies with butoconazole 1-BSR indicated treatment-related urogenital irritation in 1% of patients and irritative symptoms with an unknown or unspecified relationship to the study drug in 2.5% of patients. The leakage was insignificant.[10]

In conclusion, under carefully controlled study conditions, this clinical trial established the therapeutic equivalency of butoconazole 1-BSR and miconazole 7. In addition, the trial demonstrated a significant difference in favor of butoconazole 1-BSR in relief of severe symptoms on the first posttreatment day. Ensured compliance resulting from only a single-application regimen, efficacy and highly acceptable safety profile support the use of butoconazole 1-BSR for the management of vulvovaginal candidiasis in clinical practice.

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Exhibit 2

1: Expert Opin Pharmacother. 2002 Aug;3(8):1059-65.

Treatment of vaginal Candida infections.

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Candida vaginitis is most commonly caused by Candida albicans (> 85%) with little evidence of an increase in vaginitis due to non-C. albicans species. Epidemiological studies are no longer possible in the US in the era of self-diagnosis and -treatment by women empowered by the availability of over-the-counter antimycotics. A new classification of vulvovaginal candidiasis into uncomplicated and complicated vaginitis has simplified choice and duration of antifungal therapy. Vaginitis due to C. albicans responds well to available therapy. In contrast, vaginitis due to Candida glabrata is associated with a high treatment failure rate. Candida vaginitis infection rates in HIV-positive women remain undetermined and reports of refractory fungal vaginitis have not been substantiated. In spite of the wide array of antifungal agents currently available, considerable limitations in available therapy exist in the effective management of complicated vaginitis.

PMID: 12150685 [PubMed - indexed for MEDLINE]

Exhibit 3

1: Semin Respir Infect. 2002 Jun;17(2):91-8.

Links

Non-albicans Candida infections in patients with hematologic malignancies.

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The epidemiology of invasive Candida infections is characterized by a recent decline in Candida albicans and C. tropicalis with a concomitant increase in the incidence of non-albicans Candida species such as C. glabrata and C. krusei, a phenomenon at least partly ascribed to triazole use. Of particular concern is the recognition that these organisms are multiresistant, adding a new dimension to the challenge of management. C. lusitaniae, though uncommon, mainly presents as breakthrough fungemias in patients on antifungal therapy. C. parapsilosis, an organism of relatively low virulence, plays an increasing role because of its ability to cause intravenous line and hyperalimentation fluid colonization. Emergence of azole-resistant (relative or absolute) Candida species is being met by a new class of antifungal agents namely the echinocandins and a new generation of broad-spectrum triazole agents. This article addresses the biology, epidemiology, prognostic factors, and management of an emerging group of pathogens such as non-albicans Candida.

PMID: 12070827 [PubMed - indexed for MEDLINE]

1: Am J Obstet Gynecol. 2001 Aug;185(2):363-9.

Treatment of complicated Candida vaginitis: comparison of single and sequential doses of fluconazole.

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OBJECTIVE: An attempt was made to validate recent recommendations that women with complicated Candida vaginitis (severe or recurrent, non-albicans Candida spp or abnormal host) require longer-duration antifungal therapy to achieve clinical cure and mycologic eradication. STUDY DESIGN: A prospective, multicenter, randomized, double-blind study was performed comparing a single dose of 150 mg of fluconazole with 2 sequential 150-mg doses of fluconazole given 3 days apart. RESULTS: Five hundred fifty-six women with severe or recurrent Candida vaginitis were enrolled, and 398 had at least one postbaseline evaluation (intent to treat) and of these 309 were fully evaluable (efficacy-valid). At baseline, 92% of vaginal isolates were Candida albicans. The 2-dose fluconazole regimen achieved significantly higher clinical cure rates in women with severe vaginitis when evaluated on day 14 (P =.015) and higher clinical and mycologic responses persisted at day 35. Women with recurrent but not severe vaginitis did not benefit clinically short term by the additional fluconazole dose. Multivariate logistic regression analysis showed that being infected with non-albicans Candida predicted significantly reduced clinical and mycologic response regardless of duration of therapy. Fluconazole therapy was well tolerated and free of serious adverse effects. CONCLUSION: Treatment of Candida vaginitis requires individualization, and women with severe Candida vaginitis achieve superior clinical and mycologic eradication with a 2-dose fluconazole regimen.

PMID: 11518893 [PubMed - indexed for MEDLINE]